Adoptive T cell therapy: Grand Challenges and Opportunities

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Overview

- Prime-boost strategies
- Lentiviral engineered T cell transfers
General Approaches for Adoptive T Cell Therapy

A. Harvest PBMC by apheresis

B. TIL cell isolation

PB T cell transfer

T cell in vitro activation and expansion

Lymphodepleted cancer patient

± HSC

Host condition chemotherapy ± radiotherapy

TIL cell transfer

TIL cell in vitro activation and expansion

Cancer patient

J Clin Invest 2007 117:1466-76
Human T Cell Adoptive Immunotherapy (Effectors)


- 2002: Lymphodepletion + TILs + high dose IL-2 for metastatic melanoma (Dudley et al, Science 2002; 298: 850)


Cell Culture Approaches for Adoptive T Cell Therapy

Starting T cell repertoire

- TILs or PBMCs

Antigen-specific stimulation

- Antigen and APC

Time: 6 w

Polyclonal stimulation

- Cyclic stimulation with CD3- and CD28-specific antibodies

Time: 7-10 d

- Functional development
  - Treg cell depletion
  - Genetic modification
  - T cell selection and/or expansion in the host
T Cell Culture Systems

- physiologic
- pharmacologic
T Cell Artificial APC Culture System

Artificial DC: Bead

Anti-CD3 Anti-CD28

TcR/CD4

CD28

CTLA4

Signal 1

Growth

Levine et al. Science. 1996; 272:1939
Clinical Scale T Cell Culture Process

Wave Bioreactors

Combination immunotherapy: vaccination and adoptive transfer as a “prime-boost”
Multiple Myeloma

- Plasma cell neoplasm characterized by serum monoclonal Ab, osteolytic lesions, pathological fractures, anemia, hypercalcemia
- 15% of hematologic malignancies
- Autologous transplants are highly effective for tumor reduction (first line therapy), but cures are infrequent.
- GVM/GVT: Allogeneic transplants can induce cures, but treatment-related risks are high.
Mobilization

Stem Cell Collection

High-dose Melphalan

Stem Cell Transplant

Immune Assessment Studies

T Cell Infusion (Day +12)

T Cell In Vitro Activation and Expansion to Infuse $10^{10}$ Cells

Randomize

Pneumococcal Vaccine (PCV)

T Cell Collection

Mobilization

Stem Cell Collection

High-dose Melphalan

Stem Cell Transplant

T Cell Infusion (Day +12)

PCV vaccinations (Days +14, +42, +90)

Immune Assessment Studies

Adoptive transfer of vaccine primed T cells augments immunity in lymphodepleted hosts:
Summary of first trial

- First successful randomized multicenter adoptive immunotherapy trial
- Accelerated recovery of CD4 and CD8 counts to normal levels by day 42 ($P=0.004$)
- Protective antibody levels established by day 30
- Improved proliferative capacity of CD4 T cells to vaccine carrier antigen ($P<0.01$) and to Staphylococcal enterotoxin B ($P=0.004$)

=> Adoptive transfer of vaccine primed T cells appears to facilitate establishment of CD4 T central memory cells

Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells

- Eligibility
- Tumor Restaging
- Day –28 Apheresis
- Day –14 Priming Vaccine
- Day 0 Lymphodepletion Chemotherapy
- Day 2 Booster Vaccines
- Day 2 T cell transfer
- T cell in vitro activation and expansion
- Immunoassessment
Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells

PIs: Aaron Rapoport, U Maryland
Edward Stadtmauer, U Pennsylvania

INDs:
Vaccine (Vonderheide)
T cells (June)

Design: Randomized (biologic) comparison
1) Autologous T cells day 2 post ASCT
2) Vaccine + vaccine primed T cells

Status:
Protocol open to accrual
18 patients enrolled
# SUMMARY OF MYELOMA TRIAL PATIENTS

As of October, 2007

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<th>ID #</th>
<th>Age on Study</th>
<th>R</th>
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<th># hTERT VaccinesReceived</th>
<th>Day 0</th>
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hTERT vaccine + day 2 T cell trial: Interim Summary

- 18 patients enrolled
- Safety to date: no HSC engraftment issues
- Unexpected adverse event:
  - T cell engraftment syndrome in 6 patients (skin rash, fever, diarrhea)
  - Lymphocytosis: sustained in many patients
- Above implies major schedule dependent (day 2 vs day 12) difference in T cell engraftment and effector functions
**T Cell Expansion in Lymphopenic Hosts**

**Enhanced CD8 Effector Function?**

Potential mechanisms:
- Role of lymphopenia
- Depletion of Tregs, NKT, B cells?
- Removal of cytokine sinks?
  - IL-2 vs IL-7/-15/-21 regulation
- Stem cell push?

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**Day 12 p HSC**

- NKT
- APC
- Tumor
- Treg
- Teff
- iMC
- Macrophage

**Day 2 p HSC**

- APC
- Tumor
- Teff
- IL-7/15/17/21?
- HSC?

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*J Clin Invest. 2007 117:492-501*
## Rationale for Adoptive T Cell Immunotherapy with Genetically Engineered T Cells

<table>
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<tr>
<th>Natural T Cells</th>
<th>Gene-Modified T cells</th>
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<td>Safety profile established</td>
<td>Safety profile scant</td>
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<td>T cells have the potential to target cancer stem cells</td>
<td>Repertoire limitations can be overcome</td>
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<td>Anecdotal responses observed to immunotherapy</td>
<td>Anecdotal responses observed in immunotherapy</td>
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<td>Repertoire may be inadequate or lacking</td>
<td>Efficient <em>gene transfer</em> required</td>
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<td>Immunosenescence a major issue in humans</td>
<td>Efficient <em>T cell culture</em> required</td>
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Oncoretroviral vs. Lentiviral vectors

Oncoretroviral vectors

- Only transduces dividing cells
- Insertional mutagenesis
- T cell leukemia in SCID (cγ chain)

Potential advantages of HIV-based lentiviral vectors

- High efficiency transduction
- Long term expression ⇒ less susceptible to silencing
- Not yet tested in humans
First in Humans Test of Lentiviral Vectors

Levine et al. PNAS 2006

Kohn, Nat Biotech, 2007
• Objectives: determine safety and trafficking of multiple infusions of CD4 T cells transduced with anti-sense HIV
• Status
  - study opened in August 2006
  - 11 patients enrolled and 9 patients infused
Lentivector Persistence in CD4 Cells

- Long term persistence of non-selecting vector
- No cytokine support
- VSV-g immunogenicity, 4 of 7 pts to date

Multiple infusions

Single infusion:
$T_\frac{1}{2} = 23.5 \pm 7.7$ days

LOD = 200
Memory Stem Cell Hypothesis

- Studies in mice:
  - Fearon, *Science*. 293: 248
  - Zhang, *Nat Med*. 11: 1299
- Implications for human T cell gene therapy
Lessons from the First Lentiviral Gene Transfer Trial - HIV

- HIV based vectors appear safe in 13 of 13 patients treated to date
- Promising engraftment with genetically engineered CD4 T cells
- No evidence for insertional oncogenesis
- HIV based vectors have promise for HIV and cancer therapy

Levine et al. PNAS 2006
Strategies to Improve Adoptive Transfer of Tumor Specific T Cells Using Genetic Modification

Specificity
- Chimeric antigen receptors
  - Endogenous TCR
  - CD19 scFv
  - CD19
- Cloned TCR

Survival
- Costimulatory proteins
  - IL-2R
  - IL-2
- Chimeric cytokine receptors
  - GM-CSF

Localization
- Chemokine receptors
  - CCR14
- Chemokine gradient
  - Tumor

Effector Function
- Blockade of inhibitory proteins
  - Cytokines
  - Perforin


Ho, Greenberg et al, Cancer Cell (2003)
Redirected T Cell Approaches with engineered T cells
Lentiviral Redirected T Cells Targeting CD19 or Mesothelin

Anti-meso /CD19 scFv

Carmine Carpenito
Michael Milone
Mesothelin as a tumor target for EOC

- 40 kDa gpi linked surface glycoprotein that is widely expressed in EOC, mesothelioma and pancreatic cancers (Hassan and Pastan, Clin Can Res 2004)
  - Restricted expression in normal cells

- **Mesothelin cell biology**
  - Unique transcripts in serum of EOC patients (Scholler et al, PNAS 1999)
  - Mesothelin binds to Muc16/ CA125 (Rump et al, JBC 2004)

- **Mesothelin subject to immunosurveillance**
  - can be recognized by HLA class I restricted CD8 T cells (Thomas et al, JEM 2004)
  - 40% of patients with EOC have antibodies to mesothelin (Ho et al, Clin Cancer Res 2005; 129:515)
Mesothelin as a Target for Adoptive Transfer Therapy

- 40kDa gpi linked surface glycoprotein that is widely expressed in EOC, mesothelioma and pancreatic cancers
- Restricted expression in normal cells

Mesothelin cell biology
*meso*−/− mice are healthy and fertile
- Possible role in adhesion and metastasis
- Binds CA125 (muc16): large protein expressed by OvCa and normal mesothelial cells

Gubbels et al
Mol Cancer 5(1):50
Carmine Carpenito
Michael Milone

Lentiviral Redirected T Cells Targeting Mesothelin

Expression: Primary T Cells

C

non-transduced  SS1-BBz  SS1-CD28z

SS1-CD28BBz  SS1-z  SS1-Δz

FL4

FL4

SCFv-PE (FL2)

SS1-scFv

CD8α hinge

CD8α TM

CD28 TM

4-1BB

CD28

CD3ζ
Mesothelin Redirected T Cells Kill Primary Ovarian and Mesothelioma Cells In Vitro

Log fluorescence intensity

Cell counts

% lysis

E:T ratio

Carmine Carpenito
CD8 T cells expressing $\text{scFv}^{\text{meso}}$ kill $\text{pat108.gfp}$ cells at low E:T ratios

Day 0: 1 T cell added per 20 tumor cells
Day 2: Photograph
Co-stimulatory Domains Induce Cytokine Secretion in CD8 T Cells in Response to Mesothelin

Relative IL-2 mRNA expression

Meso-trunc  Meso-zeta  Meso-bb-z  Meso-28-z

CD8 T cell population
SCID-Winn Assays: Summary

T cells expressing scFv^{meso} are able to kill mesothelin^{+} tumor cells

T cells expressing scFv^{CD19} do not kill mesothelin^{+} tumor cells at E:T ratio (1:2), redirected T cells inhibit A431.meso tumor cells (serial killing)

combine 1 million A431 or A431.meso cells with varying numbers of T:scFv^{meso}Zeta and inject into opposite flanks of Rag2 \( \gamma^-/- \) mice
Human T cell Absolute Counts in Blood

CD4 T cells
CD8 T cells

A431
A431.meso

day 0: inject A431 tumor cells s.c.
day 4: inject scFv^{meso} T cells i.v.
Specific Killing of Mesothelin Tumor in NOD-SCID-β2−/− Mice: Day 4 Challenge

GFP Transduced T Cells

scFv Meso:CD28/4-1BB/CD3ζ T Cells

5 mice / gp
1x10⁶ A431 cells
0.5x10⁶ T cells day 4
Meso Redirected T Cells: In Vivo Killing of Large Established Primary Mesothelioma Xenografts: Day 45 Challenge

- saline
- gfp
- SS1-trncZ
- SS1-Zeta
- SS1-CD28z
- SS1-BBz
- SS1-tpr
- SS1-CD28z

IT injection 10e6 T bodies
N=8 mice / gp
Summary - II

• Lentiviral vectors provide an efficient means to engineer human T cells with artificial antigen receptors
• CD8+ T cells armed with mesothelin-specific T-bodies efficiently lyse mesothelin + tumor cells including primary tumor cells
• T-bodies can trigger T cell proliferation
• Addition of co-stimulatory signal transduction domains to TCR-ζ containing T bodies enhance cytokine production
• Human T cells engineered with a minimal anti-meso T-body can suppress the development of tumors in a NOD-SCID-β2-/- model of ovarian cancer
• anti-Meso T cells eradicate vascularized (45 days) xenografts in humanized NOG mice
Efficacy of anti-CD19 Lentiviral Redirected T Cells
Day 14 Challenge Model w Primary Leukemia Xenografts

- 3x10^6 gene-modified T cells/mouse
- ALL and T cell enumeration in blood performed by BD TruCount
UPenn Protocol #805313: Competitive Repopulation Strategy to Test Signaling Domains in Redirected Autologous T Cells

Leukapheresis:
patient with CD19+ leukemia or lymphoma

CD3 enrichment, parallel T body transduction and expansion

Combine RAT-19 cells during infusion

Normalize RAT-19 cell numbers

CD19-CD3ζ-4-1BB

Baseline (T=20min)

T cell genotype
- Wild type
- RAT-19
- CD3ζ-4-1BB
- RAT-19 CD3ζ

Outcomes (T=28 days)

CD19-CD3ζ-4-1BB better

OR

No difference

Monitor expansion and persistence (blood) and trafficking (bone marrow)
Conditionally Retargeting T bodies using "Cis" and "Trans" Costimulatory Domains

\[ \text{\(\alpha_{CD19}\) scFv} \quad \text{\(\alpha_{Meso}\) scFv} \quad \text{scFv} \]

TCR\(\zeta\)ta
tail

\text{Signal 1}

\text{CD28 tail}

\text{Signal 2}

\text{Signal 1 + 2 "cis"}

Chrystal Paulos
High transduction efficiency can be achieved in human CD4 T cells using lentiviral vector CD28 and TCR Zeta constructs.

Single and double transduction constructs: surface expression 67-97% efficacy.
Retention of TCR induced proliferation, and absence of autonomous proliferation Directed by "Cis" and "Trans" Costimulatory Domains

Target: Media alone

Beads alone

Naked K562

Log CD4 T cells (1e6)

Days post re-stimulation

Chrystal Paulos
Selective Proliferation Directed by “Cis” and “Trans” Costimulatory Domains

Target: CD19 K562

Mesothelin K562

CD19 K562 + Meso K562

Days post re-stimulation
Towards Personalized Medicine: T Cell Adoptive Transfer Immunotherapies

- T cells have a number of properties to be the elusive “weapon of mass destruction” for cancer and chronic infections
  - Targeting/trafficking to tumor and sites of infection demonstrated
  - Long term persistence and stem cell like qualities of central memory T cells
  - Strategies to enhance function of T cells by genetic engineering
- Barriers to widespread utilization
  - Efficient T cell culture systems
  - Efficient T cell engineering
Enthusiasm for T Cell Gene Therapy

Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity
(chimeric genes/antibody variable region)

ADA Correction

↓ Engraftment
↑ Trafficking

X-SCID (cγ chain) Correction

Jesse Gelsinger

NCI TCR Trial

? Lentiviral Vectors

Lentiviral Vectors

High

None

Some

1989
1995
1997
1999
2001
2002
2006
Collaborators and Acknowledgements

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Megan Suhoski
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NCI
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Leukemia & Lymphoma Society
Alliance for Cancer Gene Therapy